

Amendments to the Specification:

Please replace the paragraph on page 120, lines 1-5, with the following amended paragraph:

Example 1 - Preparation and biological evaluation of multiple-specific gas-containing microbubbles of DSPS ‘doped’ with a lipopeptide consisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and a fibronectin peptide (WQPPRARI) SEQ ID NO:2.

Please replace the paragraph on page 120, lines 11-15 with the following amended paragraph::

a) Synthesis of a lipopeptide consisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and fibronectin peptide (WQPPRARI) SEQ ID NO:2.

SEQ ID NO:3

Please replace the paragraph on page 121, lines 10-13 with the following amended paragraph:

b) Preparation of gas-containing microbubbles of DSPS ‘doped’ with a multiple-specific lipopeptide consisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO: 1 and fibronectin peptide (WQPPRARI) SEQ ID NO:2.

Please replace the paragraph on page 122, lines 1-5 with the following amended paragraph:

c) In vitro study of gas-containing microbubbles of DSPE ‘doped’ with a multiple-specific lipopeptide consisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and fibronectin peptide (WQPPRARI) SEQ ID NO:2: binding to endothelial cells under flow conditions

Please replace the paragraph on page 124, lines 21-25 with the following amended paragraph:

Example 2 - Multiple-specific gas-containing microbubbles of DSPE ‘doped’ with RGDC SEQ ID NO: 4-Mal-PEG₂₀₀₀-DSPE and a lipopeptide consisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and fibronectin peptide (WQPPRARI) SEQ ID NO:2

Please replace the paragraphs beginning on page 125 line 9 and ending on page 126 line 11 with the following amended paragraphs:

b) Synthesis of RGDC SEQ ID NO:4

The RGDC SEQ ID NO:4 peptide was synthesised on a ABI 433A automated peptide synthesiser (0.25 mmol scale, Fmoc-Cys(Trt)-Wang resin, (Novabiochem). All amino acids were activated using HBTU. The crude peptide was removed from the resin and simultaneously deprotected in TFA containing 5% EDT, 5% phenol and 5% water. Following evaporation of the excess cleavage solution the peptide was precipitated and triturated several times with diethyl ether before air drying. The crude peptide was purified by preparative hplc and fractions containing pure product combined and freeze dried. Final characterisation was performed using analytical hplc and MALDI MS.

c) Preparation of multiple-specific gas-filled microbubbles encapsulated by phosphatidylserine and ‘doped’ with RGDC SEQ ID NO:4-Mal-PEG₃₄₀₀-DSPE and a lipopeptide comprising a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and fibronectin peptide (WQPPRARI) SEQ ID NO:2.

DSPS (Avanti, 5.0 mg), lipopeptide (0.5 mg) from example 1 a) and PE-PEG-MAL (0.5 mg) from section a) was weighed into a clean vial and 1.0 mL of a solution of 1.4% propylene glycol/ 2.4% glycerol added. The mixture was sonicated for 3-5 mins, warmed to 80°C for 5 minutes then filtered through a 4.5 micron filter. The mixture was cooled to room temperature and the head space flushed with perfluorobutane gas. The vials were shaken in a cap mixer for 45 s and the microbubbles centrifuged at 1000 rpm for 3 minutes. The infranatant was exchanged with 1 mL of PBS containing 1 mg of the peptide RGDC SEQ ID NO:4 and the pH adjusted to 8. The conjugation reaction was allowed to proceed for 2 h. The bubbles were washed in PBS then with water until all unreacted RGDC SEQ ID NO:4 had been removed from the infranatant as observed by MALDI-MS. The microbubbles were further analysed by Coulter counter (98% between 1 and 7 micron).

Page 128, line 4 insert the following line

SEQ ID NO:5

Page 128, line 8 insert the following line

SEQ ID NO:5

Page 128, line 34 insert the following line

SEQ ID NO:5

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Please replace the paragraphs beginning on page 129 line 35 and ending on page 130 line 5 with the following amended paragraphs:

Example 5 - Multiple-specific gas-containing microbubbles encapsulated with phosphatidylserine and biotin-PEG₃₄₀₀-alanyl-cholesterol and functionalised with streptavidin/biotinyl-endothelin-1 peptide (biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH) SEQ ID NO:6 and biotinyl-fibrin-anti-polymerant peptide (biotin-GPRPPERHQ.S.NH₂) SEQ ID NO:7

Please replace the paragraph on page 130, lines 24-25 with the following amended paragraph:

b) Synthesis of biotinylated endothelin-1 peptide (biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH) SEQ ID NO:6

Please replace the paragraph on page 131, lines 14-15 with the following amended paragraph:

c) Synthesis of biotinyl-fibrin-anti-polymerant peptide (Biotin-GPRPPERHQ.S.NH₂) SEQ ID NO:7

Please replace the paragraph on page 132, lines 18-26 with the following amended paragraph:

Example 6 - Multiple-specific gas-filled microbubbles encapsulated with phosphatidylserine and a biotinylated lipopeptide used to prepare a streptavidin 'sandwich' with a mixture of

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biotinyl-endothelin-1 peptide (biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH) SEQ ID NO:6 and
biotinyl-fibrin-anti-polymerant peptide (biotin-GPRPPERHQ.S.NH₂) SEQ ID NO:7

a) Synthesis of lipopeptide dipalmitoyl-lysyl-tryptophanyl-lysyl-lysyl-lysyl(biotinyl)-glycine SEQ ID NO:8

Please replace the paragraphs beginning on page 133 line 33 and ending on page 134 line 3 with the following amended paragraphs:

c) Preparation of multiple-specific gas-filled microbubbles encapsulated with phosphatidylserine and a biotinylated lipopeptide and functionalised with streptavidin/biotinyl-endothelin-1 peptide (biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH) SEQ ID NO:6/biotinyl-fibrin-anti-polymerant peptide (biotin-GPRPPERHQ.S.NH₂) SEQ ID NO:7

Please replace the paragraph on page 134, lines 9-14 with the following amended paragraph:

Example 7 - Multiple-specific gas-filled microbubbles encapsulated with phosphatidylserine and biotin-DPPE used to prepare a streptavidin ‘sandwich’ with a mixture of biotinyl-endothelin-1 peptide (biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH) SEQ ID NO:6 and biotinyl-fibrin-anti-polymerant peptide (biotin-GPRPPERHQ.S.NH₂) SEQ ID NO: 7

Please replace the paragraph on page 134, lines 27-32 with the following amended paragraph:

b) Conjugation of gas-filled microbubbles encapsulated with phosphatidylserine and biotin-DPPE with streptavidin and a mixture of biotinyl-Endothelin-1 (biotin-D-Trp-Leu-Asp-Ile-

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Ile-Trp.OH) SEQ ID NO:6 and biotinyl-Fibrin-anti-polymerant peptide (biotin-GPRPPERHQSNH₂) SEQ ID NO:7

Please replace the paragraph on page 136, lines 22-26 with the following amended paragraph:

Example 9 - Multiple-specific gas-filled microbubbles encapsulated with phosphatidylserine/streptavidin-Succ-PEG-DSPE and the oligonucleotides biotin-GAAAGGTAGTGGGGTCGTGTGCCGG SEQ ID NO:9 and biotin-GGCGCTGATGATGTTGTTGATTCTT SEQ ID NO:10

Please replace the paragraphs on page 137, lines 6-24 with the following amended paragraphs:

d) Preparation of gas-filled microbubbles encapsulated with phosphatidylserine/streptavidin-Succ-PEG-DSPE and the oligonucleotides biotin-GAAAGGTAGTGGGGTCGTGTGCCGG SEQ ID NO:9 and biotin-GGCGCTGATGATGTTGTTGATTCTT SEQ ID NO:10

Microbubbles from section c) are incubated in a solution containing a mixture of biotin-GAAAGGTAGTGGGGTCGTGTGCCGG SEQ ID NO:9 and biotin-GGCGCTGATGATGTTGTTGATTCTT SEQ ID NO:10. The oligonucleotide-coated microbubbles are washed as described above. Binding of the oligonucleotide to the bubbles is detected e.g. by using fluorescent-labeled oligonucleotides for attachment to the bubbles, or by hybridising the attached oligonucleotide to a labeled (fluorescence or radioactivity) complementary oligonucleotide. The functionality of the oligonucleotide-carrying

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microbubbles is analysed, e.g. by hybridising the bubbles with immobilized DNA-containing sequences complementary to the attached oligonucleotide.

Please replace the paragraph on page 139, lines 1-5 with the following amended paragraph:

Example 11) Preparation of multiple-specific hollow polymer particles incorporating avidin in the polymer wall conjugated with the oligonucleotide biotin-GGCGCTGATGATGTTGATTCTT SEQ ID NO:10 and the endothelin-1 peptide biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH SEQ ID NO:6

Please replace the paragraph on page 140, line 3 with the following amended paragraph:

b) Synthesis of biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH SEQ ID NO:6

Please replace the paragraph on page 140, lines 10-15 with the following amended paragraph:

The particles from a) were centrifuged and the supernatant replaced with 1 mL of PBS buffer pH 7.5 containing 0.2 mg of biotin-GGCGCTGATGATGTTGATTCTT SEQ ID NO:10 and 0.2 mg of biotin-D-Trp-Leu-Asp-Ile-Ile-Trp.OH SEQ ID NO:6 from b) above. After incubation for 24 h the particles were washed extensively with PBS and water

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Please replace the paragraph on page 142, lines 4-7 with the following amended paragraph:

a) Synthesis of a lipopeptide containing the RGD sequence and a fluorescein reporter group:
Dipalmitoyl-Lys-Lys-Lys-Lys[Acetyl-Arg-Gly-Asp-Lys(Fluorescein)]Gly.OH SEQ ID NO:11

Please replace the paragraph on page 144, lines 3-5 with the following amended paragraph:

a) Synthesis of an endothelial cell binding lipopeptide: 2-n-hexadecylstearyl-Lys-Leu-Ala-Leu-Lys-Leu-Ala-Leu-Lys-Ala-Leu-Lys-Ala-Ala-Leu-Lys-Leu-Ala-NH₂ SEQ ID NO:12.

Please replace the paragraph on page 146, lines 20-21 with the following amended paragraph:

a) Synthesis of a thiol functionalised lipid molecule: Dipalmitoyl-Lys-Lys-Lys-Aca-Cys.OH SEQ ID NO:13

Please insert the following line on page 147, line 1

SEQ ID NO:13

Please replace the paragraph on page 149, lines 33-34 with the following amended paragraph:

a) Synthesis of a lipopeptide functionalised with captopril SEQ ID NO: 14:

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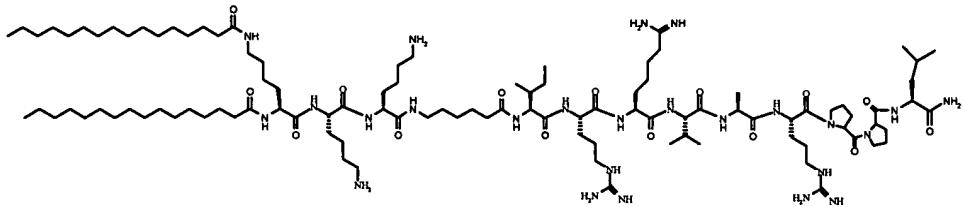
Please insert the following line on page 50, line 1

SEQ ID NO: 14

Please replace the paragraph on page 151, lines 1-3 with the following amended paragraph:

b) Synthesis of a lipopeptide with affinity for endothelial cells: Dipalmitoyl-Lys-Lys-Lys-Aca-Ile-Arg-Arg-Val-Ala-Arg-Pro-Pro-Leu-NH₂ SEQ ID NO:15

Please replace the structure on page 151 line 6 with the following structure:



Please replace the paragraph on page 153, lines 8-11 with the following amended paragraph:

a) Synthesis of a lipopeptide comprising a helical peptide with affinity for cell membranes: hexadecylstearyl-Lys-Leu-Ala-Leu-Lys-Leu-Ala-Leu-Lys-Ala-Leu-Lys-Ala-Leu-Lys-Leu-Ala-NH₂ SEQ ID NO:12.

Please replace the paragraph on page 154, lines 13-15 with the following amended paragraph:

a) Synthesis of a lipopeptide comprising an interleukin-1 receptor binding peptide:

Dipalmitoyl-Lys-Gly-Asp-Trp-Asp-Gln-Phe-Gly-Leu-Trp-Arg-Gly-Ala-Ala.OH SEQ ID

NO:16

Please replace the paragraph on page 158, lines 20-22 with the following amended paragraph:

a) Synthesis of the branched peptide Dabsyl-Tyr-Arg-Ala-Leu-Val-Asp-Thr-leu-Lys-Lys(NH₂-Arg-Gly-Asp-Ser)-Gly-Cys.OH SEQ ID NO:17

Please replace the paragraph on page 162, lines 20-25 with the following amended paragraph:

Example 23) Preparation of multiple-specific gas-containing microbubbles encapsulated with DSPS and Biotin-PEG₃₄₀₀-acyl-phosphatidylethanolamine and functionalised with streptavidin, oligonucleotide biotin-GAAAGGTAGTGGGGTCGTGTGCCGG SEQ ID NO:9 and biotinylated fibrin-anti-polymerant peptide (Biotin-GPRPPERHQ.S.NH₂) SEQ ID NO:7.

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Please replace the paragraphs on page 164, lines 4-14 with the following amended paragraphs:

c) Conjugation of streptavidin coated microbubbles with the oligonucleotide biotin-GAAAGGTAGTGGGGTCGTGTGCCGG SEQ ID NO:9 and biotinylated fibrin-anti-polymerant peptide biotin-GPRPPERHQS SEQ ID NO:7

The particles from aliquot no. 6 above were centrifuged and the supernatant replaced with 1 mL of PBS buffer pH 7.5 containing 0.2 mg of biotin-GAAAGGTAGTGGGGTCGTGTGCCGG SEQ ID NO:9 and 0.2 mg of biotin-GPRPPERHQS SEQ ID NO:7 (example 5 c). After incubation for 24 h the particles were washed extensively with PBS and water.

Please replace the paragraph on page 164, lines 30-32 with the following amended paragraphs:

a) Synthesis of a lipopeptide with affinity for thrombi (Diplamitoyl-Lys-Asn-Asp-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln.NH₂) SEQ ID NO:18.

Please insert the following line on page 165, line 1:

SEQ ID NO:18

Please replace the paragraphs beginning on page 166 line 35 and ending on page 167, line 9 with the following amended paragraphs:

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Example 25 - Multiple-specific PFB gas-filled microbubbles encapsulated with DSPS and a lipopeptide comprising a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and a fibronectin peptide (WOPPRARI) SEQ ID NO:2 for targeting and a lipopeptide containing atenolol for therapeutic applications

a) Synthesis of a lipopeptide consisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and a fibronectin peptide (WOPPRARI) SEQ ID NO:2

Please replace the paragraph on page 169 line 12-14 with the following amended paragraphs:

c) Synthesis of a lipopeptide functionalised with atenolol SEQ ID NO:19

SEQ ID NO:19

Please replace the paragraph on page 170 line 11-14 with the following amended paragraphs:

d) Preparation of gas-filled microbubbles of DSPS comprising a lipopeptide cosisting of a heparin sulphate binding peptide (KRKR) SEQ ID NO:1 and a fibronectin peptide (WOPPRARI) SEQ ID NO:2 and a lipopeptide containing atenolol

Please replace the paragraph on page 178 line 35 with the following amended paragraphs:

c) Synthesis of cholanic acid thiol ester of captopril SEQ ID NO: 20

SEQ ID NO:20

Please insert the attached nine (9) page printed version of the “Sequence Listing” into the specification as required at the end of the written description and before the claims.